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The Effect of *Candida Albicans* Systemic Infection on Matrix Metalloproteinases in Breast Cancer Bearing Balb/c Mice

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ABSTRACT

Breast cancer patients are susceptible to infections such as candidiasis. Due to the importance and the role of matrix metalloproteinases (MMP) in breast cancer progression and its correlation with tumor metastasis, we analyzed the serum level of MMPs -2, -3, -9 and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in breast cancer bearing mice in the presence of systemic *Candida albicans* infection.

Female BALB/c mice were divided into 4 groups: group I had tumor + candidiasis; group II, tumor only; group III, candidiasis only and group IV as negative control. Tumor tissue was separated from stock breast cancer bearing mice and transplanted subcutaneously into the groups I and II mice. Two weeks after tumor transplantation, groups I and III were infected with *Candida albicans* by intravenous injection. One week after systemic infection, the sera of the experimental groups were prepared and analyzed with ELISA for MMP-2, -3, -9 and TIMP-1 levels.

The results showed that the levels of MMP-3, MMP-9 and TIMP-1 were increased in groups I, II and III, as compared to the control group. However, the level of MMP-2 was decreased in mice infected with *Candida albicans* and in infected mice bearing tumor.

These data suggest that candidiasis may have a positive effect on tumor progression and metastasis.

Keywords: Breast cancer; *Candida albicans*; Candidiasis; Matrix metalloproteinase (MMP); Metastasis; Tissue inhibitor of matrix metalloproteinase (TIMP)

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INTRODUCTION

Candida (C.) albicans, a commensal of the human digestive tract, is frequently responsible for the systemic infections in immunocompromised patients.[1] Systemic *C. albicans* infection has an influence on the outcome of cancer and AIDS in patients and is responsible for the prolonged hospital stays, high healthcare costs and significant mortality.[2,3]

Breast cancer is the most common malignancy in females. In the United States of America, breast cancer is the second leading cause of female death.[4] Screening with treatment has lowered breast cancer mortality. Identification of prognostic tumor markers is a main strategy for planning treatment and predicting outcome of patients with various malignancies.[5] Recently, among markers such as tumor size, HER-2/neu gene amplification, overexpression of Bcl-2 or p53, or BRCA1 and BRCA2 mutations, the level of active matrix metalloproteinases (MMPs), especially MMP-2, is also considered to be a breast cancer metastasis indicator.[6]

Since axillary lymph node is considered to be an important place for breast cancer metastasis, the measurements of the active MMP-2 level in this area could provide important information necessary for better treatment.[7] Human MMPs are a family of over 20 different endopeptidases that are able to degrade various components of the extracellular matrix.[8] The increased expression of MMP-2, -3 and -9 proteins is linked to all human cancers[9] and correlates with worse prognosis.[10]

The ratio of MMP-9/MMP-2 was enhanced in cancer patients as compared to those with benign diseases and healthy individuals.[11] It was shown that the increased expression of MMP-3 in breast cancer patients could be predictive of tumor progression leading to metastasis.[12] MMPs are secreted as pro-enzymes, which are activated by proteolytic cleavage and regulated by a family of inhibitors (tissue inhibitors of matrix metalloproteinases; TIMPs). Therefore, MMP activities are dependent on the balance between MMP production and activation and local TIMP levels. In breast tumor tissue, the expression of TIMP-1 was enhanced when compared to the benign or normal breast tissue [13,14] and high levels of TIMP-1 mRNA as well as TIMP-1 protein were demonstrated in breast cancer and other types of cancer.[13,14]

Most of our knowledge about *Candida* infections in cancer patients has been obtained from patients with hematological malignancies.[15] Since candidemia is considered to be an important complication in patients with solid tumors, we evaluated the effect of *C. albicans* infection on breast cancer metastasis markers in BALB/c mice.

MATERIALS AND METHODS

Animals

Six- to eight-week-old female BALB/c mice (20 ± 5 g) were obtained from the Pasteur Institute of Iran (Tehran, Iran). Mice were kept in plastic cages, allowed free access to water and maintained on a 12:12 h light/dark cycle. The temperature and humidity were controlled at 23±1°C and 55±10%, respectively. The study was according to the Pasteur Institute of Iran animal welfare policies. Mice were divided into four experimental groups and each group consisted of seven mice: group I, was transplanted with breast tumor and was injected with 2×10^6 *C. albicans;* group II, was only transplanted with the breast tumor; group III: was only injected with 2×10^6 *C. albicans* into the lateral tail vein and group IV, was received only PBS as negative control.

Tumor Transplantation

The adenocarcinoma breast tumor that was induced spontaneously, was a generous gift from Dr. Hassan (Dept. of Immunology, Tarbiat Modares University, Tehran, Iran) and was used as tumor stock. Tumor was removed aseptically from transplanted mice and dissected into 0.5 cm^3 pieces and washed three times with sterile PBS. Experimental mice were anesthetized with intraperitoneal injection of ketamine and xylene (10 mg/kg of body weight) and tumor pieces were transplanted subcutaneously to the right flank.

Fungal Culture and Injection

C. albicans was obtained from Mycology Department, the Pasteur Institute of Iran.

The cells were cultured in Sabouraud dextrose agar at 37°C for 48 h. The yeast cells were transferred into the fresh medium and cultured at 37°C for a further 24 h. The cells were collected by centrifugation, washed twice with PBS and then 100 µl of cells (2×10^6) were injected into the lateral tail vein of mice.[16] Vehicle control mice were injected with 100 µL PBS via tail vein. Culture of spleen and kidney cells was performed to support the *Candida* infection.

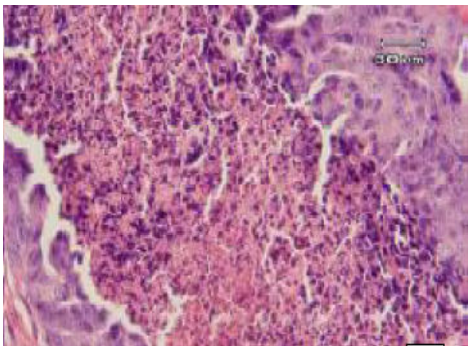

Figure 1. Pathologic slide recognizing breast adenocarcinoma in mice 3 weeks after transplantation.

Serum Collection

One week after *C. albicans* injection, blood samples were collected and sera were used for MMPs measurement.

ELISA Assay

Levels of MMP-2, MMP-3, MMP-9 and TIMP-1 were determined by ELISA at 450 nm according to the manufacturer's instructions (Quantikine, R&D Systems, USA). A standard curve using cytokine standards was constructed and the cytokine concentrations were calculated according to the standard curve.

Statistical Analysis

All the statistical analyses were conducted by SPSS 15.0 (SPSS, Chicago, IL, USA), using one-way ANOVA. The values were presented as means and standard deviations. *P* values <0.05 were considered significant.

RESULTS

Tumor Transplantation

One week after transplantation, the animals began to show tumor signs, such as increased tumor volume, aggression and body tilt. Two weeks after transplantation, tumor volume became 2-3 cm and their weight became similar. Ultimately, after 20-30 days, mice were dead. Pathologic samples were prepared for identification of breast adenocarcinoma (Figure 1).

Fungal Culture and Injection

C. albicans was cultured in corn meal agar medium plus Tween-80. The presence of yeast mycelium and chlamydoconidia proved the *Candida* genus and *Albicans* strain. For assessment of *Candida* infection, kidney and spleen cells were cultured and the pathologic slides were prepared and proved the *Candida* infection (data not shown).

MMPs ELISA Assay

One week after *C. albicans* infection, blood sample from cornea was collected and the levels of MMP-2, MMP-3, MMP-9 and TIMP-1 were determined in the sera. Figure 2 shows that in groups I, II and III, the levels of MMP-3, MMP-9 and TIMP-1 were increased as compared to the control group; however, the level of MMP-2 was decreased.

DISCUSSION

In the present study, we found that the secretion of MMP-3, MMP-9 and TIMP-1 were increased in mice bearing breast cancer tumor and the group having cancer and infected with *Candida*. Also, these proteins were increased in mice having only *C. albicans* infection. However, in infected groups (groups I and III), with or without tumor, the level of MMP-2 was decreased. Although the extracellular matrix is important in tissue development, growth, and repair,[16] the MMPs are the key components in degrading the extracellular matrix and play an important role in tumor cell evasion[17] that leads to cancer metastasis.

In breast cancer, MMP-2, -3 and -9 are thought to play an important role in invasion, metastasis and tumor angiogenesis.[13] We showed that *C. albicans* infection increases the serum level of MMP-3, -9 and TIMP-1 in mice with or without breast tumor. However, in these groups, the *C. albicans* decreased the level of MMP-2 as compared to the tumor group. It has been shown that measurement of active MMP-2 level provides important information necessary for beneficial treatment, since MMP-2 was over expressed in high-risk group patients.[7] In poor-prognosis subgroup of hormone receptor-negative patients, MMP-2 was shown to have a prognostic role in breast carcinoma.[18] Our data supported the observation of *Claveau et al* [19] that MMP-2 was decreased in *C. albicans* infected mice bearing breast tumor. MMP-2 gene activation usually does not follow by an increase in protein production. This disagreement may be explained by the fact that while *C. albicans* activates the MMP-2 gene, the protein is immediately used by the yeast as a substrate.

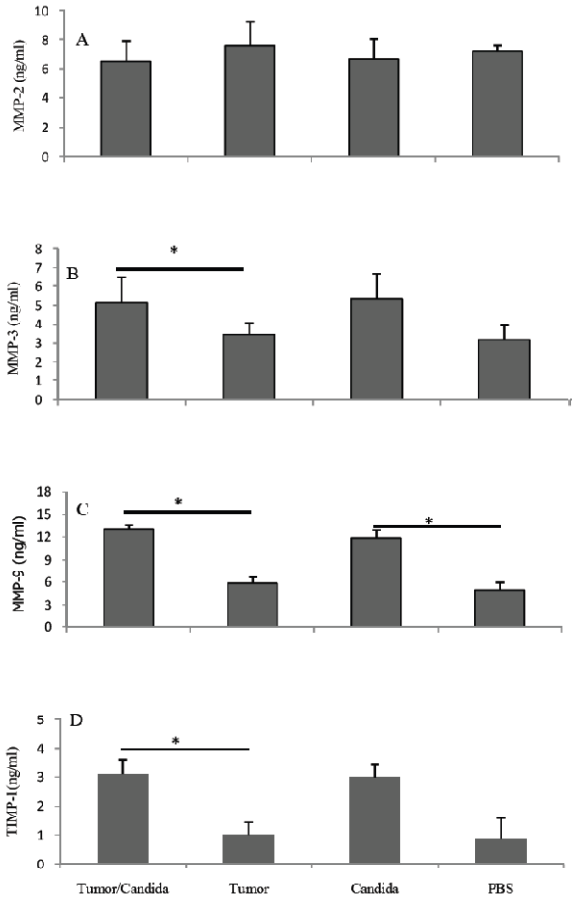

Figure 2. Analysis of MMPs and TIMP-1 in blood samples of experimental mice one week after C. albicans injection. (A), MMP-2 levels were not statistically significant in any groups (p>0.05). However, the total MMP-2 was decreased in infected mice with and without tumor as compared with "tumor only" group. (B), the differences of total MMP-3 levels between the infected groups with and without tumor were not statistically significant (p=0.337). MMP-3 levels increased in tumor group as compared to the control group, but it was not statistically significant (p=0.583). (C), Mean total MMP-9 level in all groups were increased significantly, but was not significant in tumor group as compared to the control group (p=0.015). (D), Mean total TIMP-1 level was increased significantly in tumor + Candida as compared to tumor group (p=0.000). TIMP-1 level also increased in tumor group as compared to control group, but it was not statistically significant (p=0.477). * Represents significant differences.

In a wide variety of tumor cells, MMP-9 dramatically increases; therefore, the inhibition of MMP-9 could prevent tumor progression. We show that *C. albicans* increased the MMP-9 secretion in mice bearing tumor. It seems that *C. albicans* uses MMP-9 to degrade the tissue and to disseminate[20] ; therefore, inhibition of this protease could be of interest in treating a variety of inflammatory disorders, including candidiasis. Active MMP-2 and MMP-9 were detected more frequently in malignant than benign breast carcinomas and in progressive stage in cancers.[4] Our report was in agreement with findings that the presence of both tumor and infection influenced the MMP-9 level. MMP-3 was observed in highly invasive breast cancer cell lines.[13] We found that *C. albicans* infection increased the serum level of MMP-3, but this increase was not statistically significant. The reason for this event is because we injected *C. albicans* in the early stage of tumor (before metastasis). However, in the late stage of tumor, the infected mice will die and we were not able to collect the blood samples.[21] Both tumor and host can make MMPs but their roles cannot be distinguished. Belotti et al.[22] showed that transplanted human ovarian carcinoma cell lines to mice peritoneal cavity caused secretion of high levels of host-derived MMP-9 and MMP-2 as well as human MMP-9 and MMP-2 in the ascitic fluid. However, the relative roles of host- and tumor-derived MMP-9 and MMP-2 were not defined.

Several studies have demonstrated that the expression of TIMP-1 is significantly increased in breast cancer tissue and it can be associated with a poor patient prognosis.[13] In our study, we demonstrated that TIMP-1 was increased in the presence of infection and tumor. These results showed that candidiasis probably had a positive effect on tumor progression and metastasis.

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